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Full Length Research Paper

Wound healing effects of cactus extracts on second degree superficial burned mice

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Wound healing effects of cactus extracts (CEs) were studied in this work. The aqueous extract (CAE) and ethanolic extract (CEE) each showed a slight to moderate wound healing effect on second-degree superficial burned mice. However, a combination of CAE and CEE (CME) showed great healing efficacy. At 25% concentration (w/w), CE produced highly efficient healing. The wound healing time for mice treated with CE, especially with CME, was shortened with statistical significance ($p<0.05$) relative to control mice. Granulation tissues of wounded mice treated with CE grew much better than that of wounded mice without CE treatment. Stimulation by CEs can produce relatively more blood vessels and fibroblasts at the injury sites. In addition, CE was revealed to promote blood vessel angiogenesis by up-regulating expression of vascular endothelial growth factor (VEGF). The combination of cactus CAE and CEE shows a substantial synergistic wound healing effect for second-degree burned mice. The current work also provides novel information regarding the effect of CE on accelerating the tissue-repair and wound-healing processes *in vivo* through regulation of VEGF.

Key words: Cactus extract, second-degree burn, wound healing, VEGF.

INTRODUCTION

Burn wound repair involves dynamic reciprocity between cytokine cells and extracellular matrix. The process is divided into three phases including inflammation phase, a proliferation phase and a tissue remodeling phase (Radek et al., 2005). The tissue repair and wound healing processes might be impeded by a variety of factors contributing to impaired wound healing (Singer et al., 1999). Vascular endothelial growth factor (VEGF) has proved to be a potent stimulator of migration, proliferation and survival in endothelial cells (Senger et al., 1996; Leung et al., 1989; Syridopoulos et al., 1997). VEGF is critical to proper wound-repair in stimulating angiogenesis to supply nutrients and oxygen needed for skin regrowth (Wilgus et al., 2005). The cactus extracts in this study are from the genus *Opuntia*, but we will use the common term cactus hereafter. Cactus is rich in a variety of alkaloids, flavones, glycosides and polysaccharides (Lin et al., 2002).

Cactus and cactus products have shown anti-inflammatory, immunomodulatory and anti-oxidative activities (Huang et al., 2009; Park et al., 2001; Schepetkin et al., 2008). Cactus has been used extensively in Chinese traditional medicine for treatments of lung disorders, skin diseases and blood circulation diseases. In this work, we aim to investigate the wound healing effect and mechanism of action of aqueous extract (CAE) and ethanolic extract (CEE) of cactus on second-degree burn wound healing. Surprisingly, the mixture of CAE and CEE (CME) showed a much better healing effect than individual CAE and individual CEE. The wound repair process of second-degree burned mice was significantly shortened following CME treatments. VEGF protein was up-regulated in response to CE treatment by accelerating blood vessel angiogenesis in wound repair process.

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MATERIALS AND METHODS

Plant material and experimental animal

Wild cacti of the *genus Opuntia dillenii Haw* were obtained from Shantou, China. SPF mice with body mass of 22.0 ± 2.0 g were provided by the Specific Pathogen Free Animal Laboratory of Dalian Medical University with a burn protocol approved by a committee of Liaoning Province with the approval number: (Liao) SCXK2008-0002.

Cactus aqueous extract (CAE)

The spines were removed from the wild cactus. 300 g of cactus were then minced and homogenized. Aqueous extraction was performed by soaking the homogenized cactus in 400 ml distilled water at room temperature overnight. On the next day, the supernatant was collected by centrifugation. The insoluble material was soaked in 250 ml distilled water for a second aqueous extraction. The two extracts were combined and lyophilized. The dry powder of CAE was stored at -20°C.

Cactus ethanolic extract (CEE)

The ethanolic extraction of cactus was performed according to the extraction process of CAE by using 95% ethanol instead of distilled water.

Second-degree superficial burn injury of mice

Mice were burned with a protocol (SCXK2008-0002) approved by Animal Advisory Committee of Liaoning Province to ensure the mice did not suffer excessively. Mice were anesthetized with ether and their dorsal hair was removed with an 8% (w/v) Na_2S solution. After 24 h, the mice were anesthetized again and burned with a circular pan needle (1.5 cm ID, self made) for 3 s under preheated to 99°C by electric heating in the hairless area thus producing second-degree superficial scald.

Cactus extract treatment

CAE and CEE powders were dissolved in saline. The mice with superficial second degree burn injury were randomly divided into eleven groups with twelve mice per group. Then the mice were treated with low dosage (6.25%, w/w), median dosage (12.5%, w/w) and high dosage (25%, w/w) of CAE, CEE and CME (a 1:1 mixture of CAE and CEE) once a day. The healing effects of cactus extracts on mouse skin wound recovery were observed and compared. A complete healing is defined by all the scabs falling off from the injury site. Mice treated with saline served as control mice. Additional controls were mice treated with a drug called Jingwanhong (JWH), commonly used in China for burn wound treatment. The wound scalds were observed daily following application of the cactus extracts.

Histological and pathological observation

Three mice from each group were scarified on the fifth day and twelfth day following second-degree superficial burn injury. The granulation tissues were obtained by normal resection, cut as 7 µm serial sections, fixed in peraformaldehyde, stained with hematoxylin and eosin. The histological changes were visualized with a light microscope. Following the cactus extract and drug treatment, the selected sections were scanned at 200× and 400× magnification for visualizing the number of blood vessels, fibroblasts and fibrocytes.

Western blot analysis

Three mice from each group were scarified on the third and seventh days following treatment. Then the wound regions of skin were resected, minced and sonicated in ice-cold Tris-HCl buffer (0.01 M, 0.9% NaCl, 0.2 mM EDTA, 0.1 mM DTT, pH 7.4). The supernatants were collected by centrifugation at 10000 g for 15 min at 4°C. Protein concentrations of the samples were determined by a Bradford assay for Western blot analysis. Equivalent amounts of protein from each samples were resolved by 15% SDS-PAGE and transferred onto nitrocellulose membranes. β-actin (Boston Co., Ltd, USA) was used as the internal standard. Membranes were washed three times for 10 min with PBS-T buffer (0.5% Tween-20) and blocked in nonfat milk (5%) PBS-T for 1 h at room temperature. After extensive washing with PBS-T buffer, the membranes were then incubated with VEGF antibody (Boston Co., Ltd, Wuhan, China) with shaking overnight at 4°C. Finally, the membranes were incubated with goat anti-rabbit IgG (H+L)-HRP (Boston Co., Ltd, USA) at room temperature for 2 h. The protein bands detected by antibodies were visualized by DAB staining and evaluated by Quantity One 4.4 software (Bio-RAD, USA).

Statistical analysis

Data were expressed as mean ± SD. SPSS 11.5 software was used for all statistical analysis of data. One-way ANOVA analysis of variance was used to determine the significance in two comparisons. Statistical significance was set at $p<0.05$.

RESULTS AND DISCUSSION

The combination of CAE and CEE shows a synergistic healing effect on second-degree superficially burned mice

Cactus and cactus products have shown great anti-inflammatory, immunomodulatory, anti-cancer, and anti-oxidative activities (Alarcon-Aguilar et al., 2003; Huang et al., 2009; Ji et al., 2005; Li et al., 2005; Park et al., 2001; Schepetkin et al., 2008; Wang et al., 2004; Zou et al., 2005). However, no studies have been published regarding its healing effect on second-degree superficial burn wounds.

The mixture of CAE and CEE began to show a clear burn wound repair effect on the burn-wounded mice in 48 h. The skins of injured mice were swollen obviously. And the wounds from all mice were scabbed and dried three days after the injury. Figure 1 shows the healing times for mice from different groups. The natural healing time for burn-wounded mice was 16.7 ± 0.3 d. The healing time of Jingwanhong (JWH) treated mice was 15.0 ± 0.6 d (Figure 1). The healing times for the mice treated with 6.25, 12.5 and 25% CAE were 16.8 ± 0.5 d, 16.1 ± 0.7 d, 15.9 ± 0.3 d. For the mice treated with 6.25, 12.5and 25% CEE were 16.0 ± 0.7 d, 15.7 ± 0.3 d and 15.2 ± 0.2 d. The 1:1 mixture of CAE and CEE showed strong effects

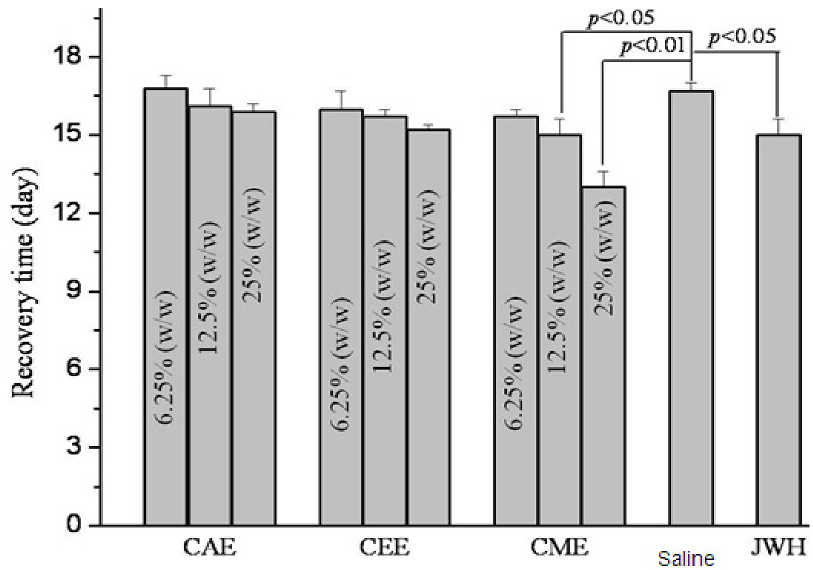

Figure 1. The wound healing effects of CAE, CEE and CME on second-degree superficial burned mice and the comparative analysis with JWH drug treatment. The notes of saline and JWH represent that the mice were treated with saline and Jingwanhong a popular Chinese traditional medicine drug commonly used in China for burn wound treatment.

on skin burn wound repair. The healing time for the mice treated with 6.25, 12.5 and 25% CME were 15.7 ± 0.3 d, 15.0 ± 0.6 d and 13.0 ± 0.6 d, respectively (Figure 1).

The results indicated that CAE showed slight healing effect. CEE with the concentration of 25.0% (w/w) could shorten the recovery time by 1.5 d. However, CME showed significant burn wound healing effect. It could decrease the healing time for second-degree superficial burn wounded mice by 22% (3.7 d, 33 replicate samples) comparing to the mice only treated with saline. CME even showed better recovery effect than JWH that is a popular drug commonly used in China for burn wound treatment (Figure 1). The CME with the concentration of 25% (w/w) could shorten the recovery time for burn wounded mice by 2 d comparing to JWH-treated mice. The above results clearly indicated that the mixture of CAE and CEE with a ratio of 1:1 in w/w unit showed highly improved healing effect on second-degree superficial burn injury mice comparing to individual CAE and individual CEE. It reveals that a magic synergistic correlation existing between CAE and CEE for accelerating burn wound healing process.

Pathological investigation of burn-wounded skins following treatments of cactus extracts

On the fifth day after injury, the majority of burn surface area sections of mice treated with 6.25% CAE were necrotic with slightly infiltrated dermal congestion. For the mouse treated with 12.5% CAE, its derma was still congested with increased neutrophil. However, for 25% CAE treated mice, reduced congestions and increased neutrophil were observed. As for 6.25% CEE treated mice, the scalded area had a large number of necrotic exudates with dermal congestion and edema. Dermal congestion was mitigated in 12.5% CEE treated skin section. While for the 25% CEE treated mice, dermal congestion and edema were observed disappeared and small granulation tissue appeared. Figure 2A indicated that on the fifth day following 6.25% CME treatment, the organization had no congestion and edema. A small amount of granulation tissue appeared. Following the increase of CME concentration, the granulation tissues were observed increased significantly (Figures 2B and C). Most of the scalded skins of burned group mice were necrotic with severe congestion and edema. In most of scalded skins treated with JWH, congestion, edema and mild inflammatory reaction were observed.

On the twelfth day following 6.25% CAE treatment, the skin sections of wounded mice showed skin necrosis, subcutaneous abscess formation and inflammatory granulation tissue. Inflammatory granulation tissue and slight skin regeneration were observed for 12.5% CAE treated mice. Small granulation tissue began to appear for 25% CAE treated mice. For 6.25% CEE treated mice, granulation tissue proliferation apparently began to replace the exudates. For the wounded mice treated with 12.5% and 25% CEE, apparent proliferation of granulation tissue replaced the exudates. For burned mice treated with 6.25% CME, inflammatory granulation tissue and skin regeneration were observed (Figure 2D).

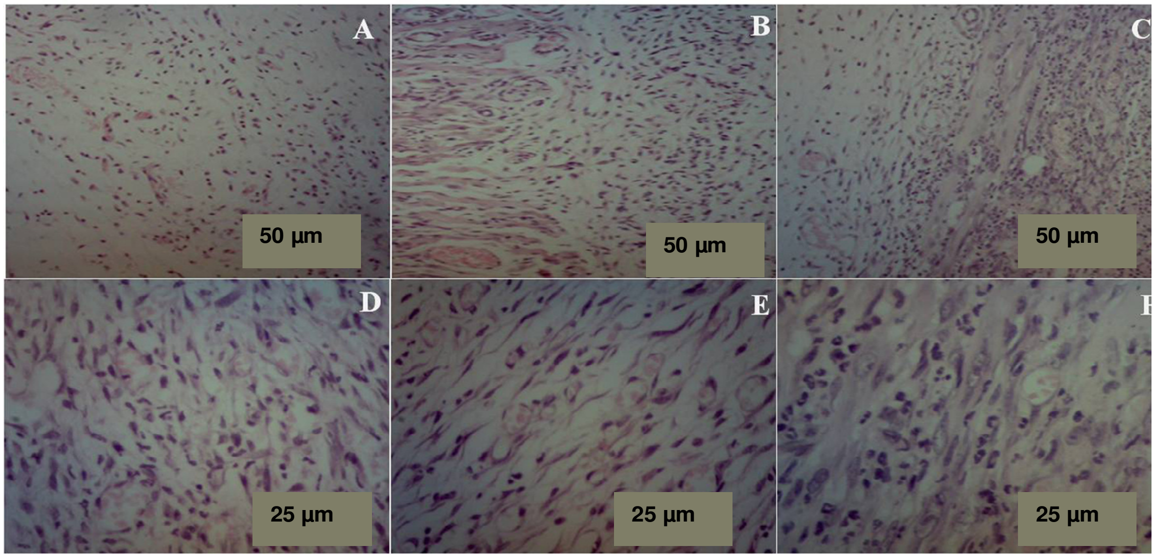

Figure 2. Histological investigations of the burn-wound healing effects of CME on mice burn-wounded skin. Plots 2A, 2B and 2C show the photographs of wounded-skins of mice following the treatments of 6.25, 12.5 and 25% CME on the fifth day. Images are obtained with a magnification of 200×. And plots 2D, 2E and 2F show wounded-skin photographs of mice following the treatments of 6.25, 12.5 and 25% CME on the twelfth day. Images are acquired with a magnification of 400×.

Table 1. Effect of cactus extract on granulation tissue at the 5th day.

Treatment	Vascular number*	Fibroblast content (%)*
6.25% (CEE)	2.40±1.36	21.45±2.72
12.5% (CEE)	4.80±1.46	19.57±2.17
25% (CEE)	4.80±0.97	17.82±2.50
6.25% (CAE)	6.00±1.22	24.47±1.05
12.5% (CAE)	6.80±0.86	24.81±5.29
25% (CAE)	6.40±1.44	15.19±2.20
6.25% (CME)	4.40±0.68	21.83±2.66
12.5% (CME)	8.40±0.51	18.54±2.11
25% (CME)	10.40±0.68	17.00±2.36
Saline	3.00±0.63	11.53±0.60
JWH	4.60±1.17	15.19±2.20

* The results were represented as the mean ± SD obtained from 33 sample replicates.

For 12.5% CME-treated injury mice, more epidermal regeneration and granulation tissue (Figure 2E) were visualized.

As for the mice treated with 25% CME, granulation tissue clearly replaced the exudates (Figure 2F) with better epithelium regeneration and regeneration of skin appendages.

For JWH-treated mice, granulation tissue and epidermal regeneration were clear. However, for the burn injured mice without treatment, only a small amount of granulation tissue was observed. No obvious skin regeneration was visible.

CAE, CEE and CME induces more blood vessels and higher content of fibroblasts

Basically, comparing to the mice treated with saline, relative higher vascular numbers and fibroblast contents were observed in granulation tissues of the mice treated either with CEE alone or with CAE alone for 5 (Table 1) days and 12 days (Table 2). And these increases were consistent and comparable with the vascular number and fibroblast content increase induced by JWH. Considering the facts that the healing effect of CAE is slightly weaker than JWH and the healing effect of CEE is comparable

Table 2. Effect of cactus extract on granulation tissue on the 12th day.

Treatment	Vascular number*	Fibroblast content (%)*
6.25% (CEE)	4.00±0.95	24.33±3.24
12.5% (CEE)	6.00±1.48	17.67±4.19
25% (CEE)	4.80±0.83	22.31±4.14
6.25% (CAE)	6.00±1.79	15.94±1.52
12.5% (CAE)	4.80±0.86	21.25±3.97
25% (CAE)	4.80±0.66	18.91±2.20
6.25% (CME)	5.20±1.46	30.63±6.35
12.5% (CME)	9.80±1.85	19.75±1.72
25% (CME)	7.00±0.32	30.18±4.67
Saline	2.40±1.44	13.26±0.84
JWH	4.80±0.86	25.95±4.07

* The results were represented as the mean ± SD obtained from 33 sample replicates.

with JWH, we might get this conclusion that more blood vessels and relative higher fibroblast content are potentially in favor of burn wound repair process. VEGF has been proved to be a potent stimulator of migration, proliferation and survival in endothelial cells (Singer et al., 1996; Leung et al., 1989; Spyridopoulos et al., 1997). It is critical to proper wound-repair by stimulating angiogenesis for supplying the nutrients and oxygen needed for skin regrowth (Wilgus et al., 2005).

Once CAE was mixed with CEE, the vascular numbers in granulation tissues flowing treatments of high concentrations of CME were found increased dramatically both at early stage and late stage of healing process comparing to the mice either treated with saline or JWH (Tables 1 and 2). And the fibroblast contents increased significantly in the CME-treated granulation tissues comparing to those of tissues of the mice treated with saline alone. In addition, the levels of fibroblast contents in granulation tissues of CME-treated mice were averaged higher than those of JWH-treated mice (Tables 1 and 2) at both early and late stages of superficial second degree burn wound repair. Taken together, the above results clearly indicated that cactus extracts potentially accelerate burn wound healing by keeping relative more blood vessels and relative higher contents of fibroblasts.

CME up-regulates VEGF expression level in burn-wounded skin

Wound repair is a process that granulation tissue gradually replaces necrotic tissue. Abnormal expressions of growth factors including VEGF, FGF and PDGF are involved in this process (Ferreira et al., 2003; Galliano et al., 2004; Judith et al., 2010; Presta et al., 2009) by affecting the nutrient substances necessarily supplied for angiogenesis. We hypothesize that cactus extract might be associated with VEGF in burn wound repair.

Protein expression levels of VEGF in wounded mice skins of different groups were examined by Western blot analysis on the third and seventh days after burn injury. Comparing to the mice treated with JWH, the VEGF levels in CME-treated mice skins on the third and seventh days were up-regulated by 149 and 132% (Figure 3), respectively, which implies that the up-regulation of VEGF protein benefits burn wound healing. The VEGF expression was significantly increased in responding to CME (12.5%) stimulation in burn-wounded mouse skin. The protein expression levels in mouse wounded skins on the third day and the seventh day following CME treatment were 2.22-fold and 1.98-fold of that in JWH-treated burn wound skin (Figure 3). Our previous results already indicated that wound repair process of second-degree burned mouse skin was significantly accelerated especially following the treatment of CME. Herein, VEGF expression was significantly up-regulated in burn-wounded mouse skin responding to CME stimulation. It is clear cactus extract up-regulates VEGF expression in mouse wounded skin, which in return stimulates angiogenesis for supplying nutrients and oxygen for skin regrowth. Consequently, a shortened healing process of second degree burn wound repair is achieved.

Conclusion

The mixture of aqueous extract and ethanolic extract of cactus (CAE and CEE) shows stronger healing effect on second-degree superficial burn injury mice than each individual one. A magic synergistic correlation exists between CAE and CEE for accelerating burn wound healing process. Cactus extracts (CEs) can alleviate the congestion of edema, reduce the necrosis of tissue at early stage of burn wound repair. CEs can also increase the proliferations of fibroblasts and neutrophils at the injury sites during the healing. Moreover, CEs promote the formations of capillaries and granulation tissues. CEs

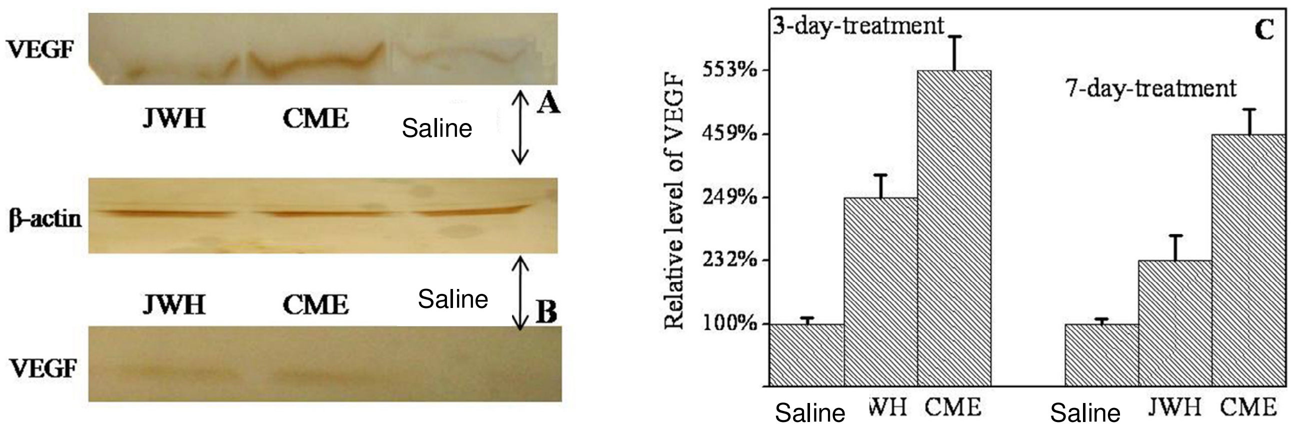

Figure 3. The expression of VEGF in 12.5% of CME treated group, saline group and JWH treated group by Western blot analysis. Plot 3A show the protein expression of VEGF on the third day in 12.5% of CME treated group, saline group and JWH treated group. Plot 3B show the expression of VEGF on the seventh day in 12.5% of CME treated group, saline group and JWH treated group. And Plot 3C shows the relative expression ratio of VEGF.

favor the regenerations of skin and appendage at late stage of burn wound recovery. More blood vessels and relative higher contents of fibroblasts induced by CEs stimulations potentially accelerate burn wound healing. We also find that cactus extract can up-regulate the expression of VEGF in wounded mouse skin. The up-regulation of VEGF benefits skin re-growth by shortening the healing process of second degree burn wound.

REFERENCES

Alarcon-Aguilar FJ, Valdes-Arzate A, Xolalpa-Molina S, Banderas-Dorantes T, Jimenez-Estrada M, Hernandez-Galicia E, Roman-Ramos R (2003). Hypoglycemic activity of two polysaccharides isolated from *Opuntia ficus-indica* and *O. streptacantha*. Proc. West Pharmacol Soc., 46: 139-142.

Ferreira LM, Gragnani A, Furtado F, Hochman B (2009).Control of the skin scarring response. An Acad Bras Cienc. Review, 81(3): 623-629.

Galiano RD, Tepper OM, Pelo CR, Bhatt KA, Callaghan M, Bastidas N, Buntin S, Steinmetz HG, Gurtner GC (2004). Topical vascular endothelial growth factor accelerates diabetic wound healing through increased angiogenesis and by mobilizing and recruiting bone marrow-derived cells. Am. J. Pathol., 164(6): 1935-1947.

Huang X, Li Q, Li H, Guo L (2009). Neuroprotective and antioxidative effect of cactus polysaccharides *in vivo* and *in vitro*. Cell Mol. Neurobiol., 29(8): 1211-1221.

Ji YB, Ji CF, Zou X, Gao SY (2005). Study on the effects of two kinds of cactus polysaccharide on erythrocyte immune function of S180 mice (in Chinese). Zhongguo Zhong Yao Za Zhi, 30(9): 690-693.

Judith R, Nithya M, Rose C, Mandal AB (2010). Application of a PDGF-containing novel gel for cutaneous wound healing. Life Sci., 87(1-2): 1-8.

Leung DW, Cachianes G, Kuang WJ, Goeddel Dv, Ferrara N (1989). Vascular endothelial growth factor is a secreted angiogenic mitogen. Sci., 246(4935): 1306-1309.

Li CY, Cheng XS, Cui MZ, Yan YG (2005). Regulative effect of *Opuntia* powder on blood lipids in rats and its mechanism. Zhongguo Zhong Yao Za Zhi (in Chinese), 30(9): 694-696.

Lin L, Bao SQ, Wang D (2002). Minireview on the researches of natural active components in *Cactaceae*. Guihaia (in Chinese), 22: 375-381.

Park EH, Kahng JH, Lee SH, Shin KH (2001). An anti-inflammatory principle from cactus. Fitoterapia, 72(3): 288-290.

Presta M, Andrés G, Leali D, Dell'Era P, Ronca R (2009). Inflammatory cells and chemokines sustain FGF2-induced angiogenesis. Eur. Cytokine Netw., 20(2): 39-50.

Radek KA, Matthies AM, Burns AL, Herinrich SA, Kovacs EJ, Dipietro LA (2005). Acute ethanol exposure impair angiogenesis and the proliferative phase of wound healing. Am. J. Physiol., 289(3): H1084-1090.

Schepetkin IA, Xie G, Kirpotina LN, Klein RA, Jutila MA, Quinn MT (2008). Macrophage immunomodulatory activity of polysaccharides isolated from *Opuntia polyacantha*. Int. Immunopharmacol., 8(10): 1455-1466.

Senger DR, Ledbetter SR, Claffey KP, Papadopoulos-Sergiou A, Peruzzi CA, Detar M (1996). Stimulation of endothelial growth factor through cooperative mechanism involving the alphabeta3 integrin, osteopontin, and thrombin. Am. J. Pathol., 149(1): 293-305.

Singer AJ, Clark RA (1999). Cutaneus wound healing. N. Engl. J. Med., 341(10): 738-746.

Spyridopoulos I, Brogi E, Kearney M, Sullivan AB, Cetrulo C, Isner JM, Losordo DW (1997). Vascular endothelial growth factor inhibits endothelial cell aptosis induced by tumor necrosis factor-alpha: balance between growth and death signals. J. Mol. Cell Cardiol., 29(5): 1321-1330.

Wang SH, Zhang DR, Yan NC (2004). Experimental studies on anti-aging actions of *Opuntia milpa alta Haw*. J. Hubei Med. Staff Coll., 17(4): 12-14.

Wilgus TA, Matthies AM, Radek KA, Dovi JV, Burns AL, Shankar K, DiPietro LA (2005). Novel function for vascular endothelial growth factor receptor-1 on epidermal keratinocytes. Am. J. Pathol., 167(5): 1257-1266.

Zou DM, Brewer M, Garcia F, Feugang JM, Wang J, Zang R, Liu H, Zou C (2005). Cactus pear: a natural product in cancer chemoprevention. J. Nutr., 4: 25.